



UNIVERSITI PUTRA MALAYSIA

**OPTIMIZATION OF PARAMETERS INVOLVED IN THE
TRANSFORMATION OF OIL PALM USING THE BIOLISTIC METHOD**

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OF OIL PALM USING THE BIOLISTIC METHOD**

By

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Thesis Submitted in Fulfilment of the Requirements for the Degree of Doctor
of Philosophy in the Faculty of Food Science and Biotechnology,
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“IN THE NAME OF ALLAH, MOST GRACIOUS, MOST MERCIFUL”

Dedicated To :

My Parents : Hj. Ghulam Kadir and Hjh. Aishan Bibi

My Wife : Nor Muriani

My Sons : Haziq, Iman and Najib

My Special Brother : Salim

My Brothers and Teachers



“By command of my Lord : Of knowledge it is only a little that is
communicated (thought) to you (O men!)”

(Surah Al-Isra' : 85)

“It is He Who produces gardens, with trellises and without, and date-palms,
and crops of diverse flavour, and the olive and the pomegranate,
similar (in kind) and different (in variety)”

(Surah Al-An'am : 141)

He Who created seven heavens in harmony. You can see no fault in the Beneficent One's
Creation; then look again: can you see any fault? Then look again and yet again; your
sight will come back to you weakened and worn out”

(Surah Al-Mulk : 3-4)



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TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	iv
LIST OF TABLES	xii
LIST OF FIGURES	xiii
LIST OF PLATES	xv
LIST OF ABBREVIATIONS	xviii
ABSTRACT	xx
ABSTRAK	xxiii
 CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	10
Genetic Transformation	10
<i>Agrobacterium</i> -mediated gene transfer	11
Advantages and Disadvantages of <i>Agrobacterium</i> -mediated Gene Transfer	15
Protoplast-mediated Gene Transfer	16
Polyethylene Glycol.....	17
Electroporation	17
Advantages and Disadvantages of Protoplast mediated Gene Transfer	17
Microprojectile Bombardment Gene Transfer	18
Advantages and Disadvantages of Microprojectile Bombardment Gene Transfer	22
Tissue Electroporation Gene Transfer	23
Silicon Carbide Gene Transfer	23
Pollen-mediated Gene Transfer	24



Advantages and Disadvantages of Pollen-mediated Gene Transfer	26
Microinjection-mediated Gene Transfer	26
Advantages and Disadvantages of Microinjection-mediated Gene Transfer	27
Ultrasonication-mediated Gene Transfer	27
Advantages and Disadvantages of Ultrasonication-mediated Gene Transfer	28
Liposome-mediated Gene Transfer	28
Laser Microbeam-mediated Gene Transfer	29
Imbibition of Seeds Gene Transfer	29
Comparison of Transformation Methods	30
Production and Confirmation of Transgenic Plants.....	31
Reporter Genes	31
Selectable Marker Genes	33
Promoter	34
DNA Integration	35
Protein Expression	35
Transmission of Transgene Into Progeny	36
Transgene Silencing	37
Strategies for Avoiding Transgene Silencing	40
Modification of Plant Oil Composition	41
<i>Brassica napus</i> (Rapeseed)	43
<i>Glycine max</i> (Soybean)	49
<i>Gossypium hirsutum</i> (Cotton)	52
<i>Zea mays</i> (Maize)	53
<i>Elaeis guineensis</i> and <i>E. oleifera</i> (Oil palms)	55
<i>Helianthus annus</i> (Sunflower)	57
<i>Arachis hypogaeae</i> (Groundnut)	58
<i>Simmondsia chinensis</i> (Jjoba)	59



	<i>Carthamus tinctorius</i> (Safflower)	59
	<i>Cocos nucifera</i> (Coconut)	59
	<i>Olea europeae</i> (Olive)	60
	<i>Linum usitatissimum</i> (Flax)	60
	Opportunitinities for genetic engineering of oil palm	61
III	MATERIAL AND METHODS	63
	Plant Materials	63
	Chemicals and Enzymes	63
	Plasmid Constructs	64
	Construction of pAP11	65
	Plasmid DNA Digestion and Fragment Isolation	67
	Ligation	67
	Preparation of Competent Cells.....	67
	Bacterial Transformation	68
	Small Scale Plasmid Isolation	69
	Large Scale Plasmid Isolation	70
	DNA-microcarrier Preparation and Bombardment for PDS- 1000/He Apparatus	71
	DNA-microcarrier Preparation and Bombardment for <i>ACCELLTM</i> Gun	73
	GUS Histochemical Assay	74
	GUS Flourometric Assay	74
	Experimental Design and Data Analysis.....	75
	Callus Initiation From Oil Palm Leaflet and Roots	75
	Callus Initiation From Oil Palm Immature Embryos	76
	Maintenance of the Embryogenic Calli	76
	Minimal Inhibitory Concentration of Selection Agents	76
	Production of Oil Palm Polyembryogenic Cultures	77
	Small Plantlet Production from Polyembryogenic Cultures ..	78



	Root initiation from Oil Palm Cultures	78
	Callus Initiation from Tobacco.....	79
	Callus Initiation from Rice Immature Embryos	79
	Selection and Regeneration of Transformants	80
	Preparation of Total DNA from Callus Culture.....	81
	Primers for PCR	81
	Purification of Primers	82
	Polymerase Chain Reaction (PCR)	82
	Southern Transfer of DNA from Agarose gel to Nylon Membrane	83
	Oligolabelling of DNA Fragments	84
	Hybridization	84
	Hygromycin Phosphotransferase (hpt) Assay.....	85
IV	RESULTS AND DISCUSSION	86
	Optimization of DNA Delivery Conditions.....	86
	Delivery of DNA into Oil Palm Cultures	86
	Removal of Endogenous GUS Background	86
	Optimization of Physical Parameters	88
	Aim : Optimization of Physical Parameters	88
	Analysis of Variance	89
	Effect of Helium Pressure	90
	Distance from Rupture Disc to Macrocarrier.....	94
	Distance from Macrocarrier to Stopping Plate.....	94
	Distance from Stopping Plate to Target Tissue.....	95
	Vacuum Pressure	97
	Effect of Bombardment Number.....	98
	Effect of Particle Type and Size.....	99
	Effect of CaCl ₂ and Spermidine on DNA-particle	



Precipitation	101
General Discussion	104
Optimized Physical Parameters	105
Optimization of Biological Parameters.....	106
Aim : Optimization of Biological Parameters.....	106
Analysis of Variance.....	106
Effect of Explant and Microcarrier Type.....	107
Effect of Time Interval from Subculture Prior to Bombardment	110
Effect of Immature Embryo Preculture Duration Prior to Bombardment	111
Effect of Post Bombardment Incubation Time.....	115
Effect of Genotype (Genetic Background).....	115
Effect of Osmoticum Types and Concentration.....	117
Effect of Pre-post Bombardment Culture Duration in 0.4M Mannitol	119
Effect of DNA Quantity Per Bombardment	121
Optimized Biological Parameters	123
Selection of Best Promoter	124
Importance of Selecting The Best Promoter for Oil Palm...	124
Selection of Best Promoter for Oil Palm	124
Selected Efficient Promoters	136
Determination of Minimal Inhibitory Concentration of Selection Agents	137
Importance of Determining The Minimal Inhibitory Concentration of Selection Agents	137
Determination of Minimal Inhibitory Concentration of selection agents	138
Effective Selective Agent	149
Aim : Optimization and Transformation Using the <i>ACCELL</i> TM Device	150



Optimization of DNA Delivery Condition for <i>ACCELL</i> TM Device	150
Selection of Transformed Callus	152
Analysis of Transformants	156
PCR Analysis	156
Southern Blot Hybridization Analysis	159
Regeneration of Transgenic Oil Palm Plants	164
Molecular Analysis of Putative Transgenic Plants	172
PCR Analysis	172
Southern Blot Hybridization Analysis	174
Rice as a Model for Monocot Transformation.....	177
Justification and Aims	177
Construction of The pAP11 Plasmid.....	178
Delivery of DNA Into Rice Immature Embryos.....	180
Selection and Regeneration of Transgenic Rice Plants.....	184
Analysis of Transgenic Plants and T1 Progenies	187
Analysis of T2 Progenies	191
 V CONCLUSION	 199
 BIBLIOGRAPHY	 203
APPENDIX	
A Schematic Representation of Transforming Plasmids.....	245
VITA	252
LIST OF PUBLICATIONS	253



LIST OF TABLES

Table		Page
1	Anova of transient <i>gusA</i> gene expression for the optimization of physical parametersn	91
2	Anova of transient <i>gusA</i> gene expression for the optimization of biological parameters	108
3	Effect of explant source on <i>gusA</i> gene expression in embryogenic calli	110
4	Effect of duration between calli transferred to medium prior to bombardment on <i>gusA</i> gene expression in embryogenic calli	112
5	Effect of duration between bombardment and GUS staining on <i>gusA</i> gene expression in embryogenic calli	116
6	Comparison of promoter strength on transient <i>gusA</i> gene expression in oil palm tissues two days after bombardment	127
7	Effect of acceleration voltage and bombardment time after subculture on transient <i>gusA</i> gene expression on oil palm callus	151
8	Effect of selection agents concentration, acceleration voltage and time for selection exposure on the recovery of resistant embryogenic callus clumps	155
9	Number of resistant embryogenic calli showing positive for amplification of <i>gusA</i> , <i>bar</i> and <i>hpt</i> genes	160
10	Summary of phenotype and genotype analysis on T1 progenies of clone T0#6	188
11	Analysis of T1 Progenies (T2) of Clone T0#6.	198
12	Summary of optimization of physical and biological parameters, promoter activity and optimal concentration of selection agents	200



LIST OF FIGURES

Figure		Page
1	Construction of pAP11 (Ubiquitin-hygromycin) plasmid for use in transformation	66
2	Effect of helium pressure (Psi) on transient <i>gusA</i> gene expression in oil palm embryogenic calli	92
3	Effect of distance from stopping plate to target tissue (cm) on transient <i>gusA</i> gene expression in oil palm embryogenic calli	96
4	Effect of microcarrier types and sizes on transient GUS gene expression in oil palm embryogenic calli	100
5	Effect of calcium chloride and spermidine in the DNA-microcarrier preparation mixture on transient <i>gusA</i> gene expression in oil palm embryogenic calli	102
6	Effect of preculture duration on transient <i>gusA</i> expression in oil palm immature embryos	114
7	Effect of type and concentration (Molar) of osmoticum sources on transient GUS expression	118
8	Effect of pre and post bombardment duration (hour) on media containing 0.4M mannitol on transient <i>gusA</i> gene expression	120
9	Effect of DNA concentration (μg) per bombardment on transient GUS expression	122
10	Comparison of transient histochemical <i>gusA</i> gene expression units in oil palm embryogenic calli and YLSP after bombardment with plasmids carrying different promoters	129
11	Comparison of transient histochemical <i>gusA</i> gene expression units in oil palm YLMP after bombardment with plasmids carrying different promoters	131
12	Comparison of transient flourometric <i>gusA</i> gene expression units in oil palm embryogenic calli and YLMP after bombardment with plasmids carrying different promoters	133



13	Comparison of transient flourometric <i>gusA</i> gene expression units in tobacco calli after bombardment with plasmids carrying different promoters	134
14	Comparison of the proliferation percentage of embryogenic calli after 30 days at different concentration of selection agents : A) kanamycin, geneticin G-418, neomycin; B) basta and C) hygromycin	139



LIST OF PLATES

Plate	Page
1: Transient <i>gusA</i> gene expression in oil palm embryogenic calli after bombardment with plasmid pEmuGN showing different efficiencies of delivery	87
2 Comparison of transient <i>gusA</i> gene expression in oil palm embryogenic calli derived from different explants after bombardment with plasmid pEmuGN	109
3 Transient <i>gusA</i> gene expression in oil palm immature embryo after bombardment	113
4 Comparison of transient histochemical <i>gusA</i> gene expression in oil palm embryogenic calli after bombardment with plasmids carrying different promoters	125
5 Comparison of transient histochemical <i>gusA</i> gene expression in oil palm YLMP after bombardment with plasmids carrying different promoters	126
6 Proliferation of oil palm embryogenic calli on media containing various concentration of kanamycin : a) control - 0 mg/l; b) 500 mg/l; c) 750 mg/l; d) 1000 mg/l, e) 1500 mg/l and f) 2000 mg/l	140
7 Proliferation of oil palm embryogenic calli on media containing various concentration of genetycin-G418 : a) control - 0 mg/l; b) 50 mg/l; c) 250 mg/l; d) 1000 mg/l, e) 1500 mg/l and f) 2000 mg/l....	141
8 Proliferation of oil palm embryogenic calli on media containing various concentration of neomycin : a)50 mg/l; b) 500 mg/l; c) 1000 mg/l, d) 2000 mg/l	142
9 Proliferation of oil palm embryogenic calli on medium containing various concentration of basta : a) control - 0 mg/l; b) 1 mg/l; c) 20 mg/l; d) 40 mg/l, e) 50 mg/l and f) 250 mg/l.....	145



10	Proliferation of oil palm embryogenic calli on media containing various concentration of hygromycin : a) control - 0 mg/l; b) 10 mg/l, c) 40 mg/l and d) 250 mg/l	146
11	Production of resistant embryogenic callus	153
12	PCR analysis of oil palm embryogenic callus (transgenic and non-transgenic) using internal control primers.....	157
13	PCR analysis of transgenic oil palm embryogenic callus.	158
14	Southern blot hybridization of transgenic oil palm embryogenic calli.....	161
15	Southern blot hybridization of transgenic oil palm embryogenic callus	163
16	Southern blot hybridization of transgenic oil palm embryogenic callus.	165
17	Production of embryos and polyembryogenic cultures from transgenic embryogenic callus	167
18	Shoots production from polyembryogenic cultures derived from transgenic embryogenic callus.	168
19	Elongation of individually isolated shoots derived from transgenic embryogenic callus	169
20	Shoot elongation and root initiation of plantlets derived from transgenic embryogenic callus	170
21	PCR analysis of transgenic oil palm plants	173
22	Southern blot hybridization of transgenic oil palm plants	175
23	Verification of pAP9	179
24	Verification of pAP11	181
25	Confirmation of pAP11	182



26	Bombardment of rice immature embryos	183
27	Hygromycin phosphotransferase assay of rice callus two days after bombardment with pAP11	185
28	Isolation of transformed callus selected on hygromycin	186
29	PCR analysis of transgenic rice clones and T1 progenies of clone T0#6	189
30	Southern blot hybridization on DNA of transgenic rice clones and progenies	190
31	Fertile transgenic T 1 plant of clone T0#6 as compared to untransformed control plant	192
32	Seeds collected from transgenic T1 plant compared to untransformed control plant	193
33	Hygromycin selection on T2 rice plants of transgenic T0#6 clone	195
34	PCR analysis of T2 progenies	197



LIST OF ABBREVIATIONS

The following abbreviations were used in the text :

ACP	Acyl carrier protein
Act1	Rice actin 1 gene's promoter
Adh1	Maize alcohol dehydrogenase 1 gene's promoter
Anova	Analysis of variance
ATP	Adenosine triphosphate
<i>bar</i>	Gene coding for phosphinothricin acetyltransferase
BSA	Bovine serum albumin
CaCl ₂	Calcium chloride
35S CaMV	Cauliflower mosaic virus 35S gene's promoter
CsCl	Cesium Chloride
2,4-D	2,4-dichlorophenoxyacetic acid
dNTP	Deoxynicotinamide triphosphate
EDTA	Ethylenediaminetetra acetic acid
Emu	A recombinant truncated maize alcohol dehydrogenase 1 gene promoter with enhancers elements from Adh1 gene and <i>Agrobacterium</i>
EtBr	Ethidium Bromide
GUS	β-Glucuronidase
<i>hpt</i>	Gene coding for hygromycin phosphotransferase
HCl	Hydrochloric acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
KAc	Potassium acetate
Kb	Kilobase
KCl	Potassium chloride
Kda	Kilodalton



KOH	Potassium hydroxide
KV	Kilovolt
LB	Luria Bertani broth
MgCl ₂	Magnesium chloride
MgSO ₄	Magnesium sulfate
MS	Murashige and Skoog
MU	Methyl umbelliferone
MUG	4-methyl umbelliferyl β-D-glucuronide
NAA	α-Naphthaleneacetic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NaPO ₄	Sodium phosphate
NH ₄ Ac	Ammonium acetate
<i>nptII</i>	Gene coding for neomycin phosphotransferase
PCR	Polymerase chain reaction
PMSF	Phenylmethylsulfonylfluoride
PVP	Polyvinyl Pyrrolidone
SDS	Sodium dodecyl sulfate
T0	First generation of transgenic plants
T1	Progeny of T0 plant
T2	Progeny of T1 plant
Tris	Tris [hydroxymethyl] aminomethane
Triton X-100	T-octylphenoxy-poly-ethoxyethanol
Tween 20	Polyoxyethylene sorbiton monolaurate
Ubi1	Maize ubiquitin 1 gene's promoter
X-gluc	5-bromo-4-chloro-3-indoyl-glucuronide
YLMP	Young leaves from mature palm
YLSP	Young leaves from seedling palm

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the Degree of Doctor of Philosophy.

**OPTIMIZATION OF TRANSFORMATION TECHNIQUES TO OBTAIN
TRANSGENIC OIL PALM**

By

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Chairman : Dr. K. Harikrishna

Faculty : Food Science and Biotechnology

Physical and biological parameters affecting DNA delivery into oil palm embryogenic calli using the biolistic device have been optimized. The physical parameters tested were : helium pressure, distance from rupture disc to the macrocarrier, distance from macrocarrier to the stopping plate, distance from stopping plate to the target tissue, vacuum pressure, number of bombardments, particle types and sizes, and the effect of calcium chloride and spermidine on microcarrier-DNA binding. The optimized biological parameters were: explant types with gold microcarrier, explant types with tungsten, duration of callus culture in fresh medium prior to bombardment, duration between bombardment and GUS staining, genotype, immature embryo preculture duration, DNA concentration, osmoticum type and concentration and osmoticum treatment duration before and after bombardment. Independent experiments were carried out to study the effects of each parameter and its



variables on transient expression. Two days after bombardment, the tissues were stained with GUS assay buffer for 16-20 hours at 37°C and the blue spots counted under a binocular microscope. All the variables used in these experiments were found to be significantly different except for vacuum pressure, bombardment number and genotype.

The efficiency of GUS gene expression was measured in embryogenic calli and young leaves of mature and seedling palms using five constructs carrying different promoters : Emu; Ubi1; Act1, 35S and Adh1 were evaluated to identify the most suitable promoter for use in oil palm. The GUS gene expression from the different promoters was assayed histochemically and fluorometrically from a total of 200 plates of target tissues in eight independent experiments. Significant effects on transient GUS gene expression were demonstrated by each of the different promoters tested.

The effectiveness of kanamycin; geneticin (G-418); neomycin, hygromycin and basta as selection agents to inhibit growth of oil palm embryogenic calli was evaluated. Embryogenic calli were separately exposed to all these selection agents at different concentrations ranging from 1 to 2000 mg/l for a period of one month. This was done in two replicates and repeated twice to ensure reproducibility of the selection system. Of the five compounds tested, hygromycin and basta were found to be most suitable as selection agents for oil palm as they can stop the growth of embryogenic calli at lower concentrations.



Bombarded embryogenic calli were exposed to 40 or 80mg/l of selective agents after 1 or 3 weeks. It was found that there were no significant differences in the number of resistant embryogenic calli produced per plate when selected at different concentrations and time. The presence of transgenes in the resistant embryogenic calli was confirmed by PCR and Southern analysis. Transgenic embryogenic calli were later regenerated into whole plants and their transgenic status verified by PCR and Southern analysis. Problems faced during the study and their solutions are also discussed.

As oil palm has a long breeding cycle, inheritance of transgenes cannot be demonstrated within the period of this study. Therefore, rice, a model crop for monocot transformation, was also used for transformation experiments. Calli derived from immature embryos were bombarded and were selected on hygromycin. Resistant calli isolated were regenerated into whole plants. Two transgenic lines were obtained. T1 and T2 from one of the clones were also produced and analysed. Integration and inheritance of the transgenes were followed by phenotypic and genotypic analysis.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk memperolehi Ijazah Doktor Falsafah.

**PENGOPTIMUMAN TEKNIK-TEKNIK TRANSFORMASI UNTUK
MEMPEROLEHI KELAPA SAWIT TRANSGENIK**

Oleh

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JANUARI 1998

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Parameter-parameter biologi dan fizikal yang mempengaruhi penghantaran DNA ke dalam kalus embriogenik kelapa sawit menggunakan alat biolistik telah berjaya dioptimumkan. Parameter-parameter fizikal yang telah diuji adalah : tekanan helium, jarak diantara cakera pecah ke pembawa makro, jarak diantara pembawa makro ke piring penghenti, jarak diantara piring penghenti ke tisu sasaran, tekanan hampagas, bilangan tembakan, saiz dan jenis pembawa mikro serta kesan kalsium klorida dan spermidin terhadap pengabungan DNA dan pembawa mikro. Parameter-parameter biologi yang telah diuji pula adalah : jenis eksplan menggunakan pembawa mikro emas, jenis eksplan menggunakan pembawa mikro tungsten, jangkamasa pengsubkulturan ke media segar sebelum tembakan, jangkamasa antara tembakan dan pewarnaan GUS, genotip, jangkamasa pra-pengkulturan embrio tidak matang



matang, kepekatan DNA, jenis dan kepekatan bahan osmotik dan jangkamasa tindakan osmotik sebelum dan selepas tembakan. Ujikaji berasingan telah dijalankan untuk mengkaji kesan setiap parameter dan pembolehubah ke atas ungkapan sementara. Dua hari selepas tembakan, tisu diwarnakan menggunakan penimbal aseii GUS selama 16-20 jam pada suhu 37°C dan bintik-bintik biru yang dihasilkan telah dikira dibawah mikroskop binokular. Setiap pembolehubah yang digunakan menunjukkan perbezaan bererti kecuali, tekanan hampagas, bilangan tembakan dan genotip.

Keupayaan ungkapan gen GUS oleh lima plasmid yang membawa promoter-promoter berbeza : Emu; Ubi1; Act1, 35S dan Adh1 telah dinilai keatas kalus embriogenik dan daun-daun muda dari pokok semaian dan pokok matang. Ini adalah untuk memilih promoter-promoter yang sesuai untuk kelapa sawit. Ungkapan gen GUS oleh promoter-promoter berbeza telah diaseii menggunakan kaedah histokimia dan fluorometrik ke atas 200 piring tisu sasaran dan di dalam 8 ujikaji berasingan. Promoter-promoter tersebut telah menunjukkan kesan yang bererti terhadap ungkapan sementara gen GUS.

Kecekapan lima agen pemilihan : kanamisin; genetisin G-418; neomisin, higromisin dan basta untuk merencat pertumbuhan kalus embriogenik kelapa sawit telah dinilai. Kalus embriogenik telah didedahkan secara berasingan kepada kepekatan berbeza (1-2000mg/l) ejen pemilihan untuk tempoh satu bulan. Ujikaji ini telah dijalankan secara replikasi dan diulang sebanyak dua kali untuk memastikan kebolehulangan hasil. Dari kelima-lima ejen pemilihan